Right-Sided Ectocervical Lesions May Be Associated with False-Negative Cytology Among Women with Histologic Cervical Intraepithelial Neoplasia 2 or 3

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■ **Abstract**

Background. The association between the location of an ectocervical lesion and the sensibility of cytologic screening has not been adequately evaluated.

Methods. We evaluated the proportion of false-negative cytologic interpretations using three independent cytologic interpretations (conventional, PapNet, and ThinPrep) according to lesion location in 111 women with histologic cervical intraepithelial neoplasia 2 or 3 of a population-based study of cervical neoplasia conducted in Guanacaste, Costa Rica. Semiquantitative measures of human papillomavirus viral load were also considered.

Results. Lesions on a women's right ectocervix were associated with more frequent false-negative results than lesions on left ectocervix for each of the cytologic methods or when the most severe interpretation was considered (p = .004). Right-sided lesions had nonsignificantly lower viral loads than left-sided lesions (p = .2).

Conclusions. Cervical intraepithelial neoplasia 2 or 3 located on the right side of the cervix may be poorly sampled with broom samplers in some settings, resulting in false-negative cytologic results. ■

Key Words: false negative, cytology, cervical intraepithelial neoplasia, human papillomavirus, cervix

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Since its introduction in the middle of the last century, cervical cytologic screening using Pap smears has reduced significantly the incidence of cervical cancer in populations where programs have been successfully implemented [1–3]. It is now recognized that Pap smears detect human papillomavirus (HPV)-induced cytomorphologic changes (e.g., low-grade squamous intraepithe-

lial lesions [LSIL] and high-grade squamous intraepithelial lesions [HSIL]) that typically precede the development of invasive cervical cancer by several years [4]. Timely detection and treatment of cancer precursors prevents the morbidity and mortality caused by cervical cancer.

Despite its effectiveness as a public health intervention, it is recognized that the Pap smear is imperfectly sensitive and that effective screening is achieved through repeated testing during the slow progression of cervical cancer precursors through several transitional states. Both negative cytologic results among women with cervical intraepithelial neoplasia (CIN; false negative) and positive cytologic results that are not a reflection of CIN (false positive) occur. The reported frequency of false-negative cytologic results varies widely [5] and is attributable to suboptimal sampling and collection of exfoliative cervical cells, slide preparation, screening, and interpretation. In recent years, efforts have been made to improve all aspects of cytologic analysis, especially collection and processing of samples, criteria for assessing samples, and nomenclature for reporting results [6–8].

To address suboptimal specimen collection, sampling devices were developed that permitted simultaneous collection of cells from the ectocervix and endocervix, assuming that many of the lesions missed by cytologic analysis were located in the endocervical canal [9, 10]. The main US classification system for reporting cervical cytologic diagnoses, the Bethesda System [7, 8], emphasizes the importance of sampling cells from the transformation zone and the endocervix for defining specimen adequacy.

Given the importance of cervical sampling for accurate cytologic evaluation, we hypothesized that topographic location of lesions could influence the performance of cytologic analysis, based on the premise that some areas of the cervix may be better sampled than others. We examined the relationship of cytologic interpretation of cervical specimens collected with Cervex brushes (Unimar, Wilton, CT) and lesion location among women with histologic CIN 2 or CIN 3 at enrollment in our population-based natural history study of HPV and cervical neoplasia in Guanacaste, Costa Rica [11, 12].

METHODS

Study Population

Subjects were participants in a National Cancer Institute-sponsored natural history study initiated in Guanacaste, Costa Rica, from 1993 to 1994. Subjects provided informed consent as required by local and US review boards [11, 12]. At enrollment, 10,049 women of 11,742 identified in a door-to-door survey of women residing in randomly chosen censal segments of Guanacaste agreed to participate in the enrollment interview. Pelvic examinations and collection of cervical specimens were performed on 9,175 women, excluding virgins (n = 583) and those women unwilling or unable to undergo a physical examination (n = 291). Details of the study design are provided elsewhere [11, 12].

Data and Specimen Collection

Participants completed a risk factor questionnaire that assessed information on sociodemographic characteristics; sexual, reproductive, and birth control practices; cigarette smoking; and histories of sexually transmitted diseases. Sexually active women underwent a pelvic examination, at which time a cervical specimen was collected with the Cervex brush used according to the manufacturer's recommendations (five sequential complete clockwise rotations). Using this sample, a conventional Pap smear was immediately prepared and fixed with Pap Perfect (MedScand, Hollywood, FL). The cells remaining on the brush were rinsed into vials containing 20 mL of preservative (PreservCyt; Cytyc Corporation, Boxborough, MA) for preparation of ThinPrep cytologic slides (Cytyc Corporation). Preparation of an additional ThinPrep slide was performed in some cases to optimize the interpretation. Then, a second specimen for HPV DNA testing [11, 12] was collected using a Dacron swab, which first was rotated inside the endocervical canal and then on the ectocervical surface. After all the samples were obtained, the cervix was washed twice with 5% acetic acid and two cervigrams were obtained.

Pathologic Analysis

Conventional smears were stained, manually screened, and interpreted locally to determine patient management. The local pathologist reviewed all smears classified as atypical squamous cells of undetermined significance (ASCUS) or more severe and 25% of those classified as the negatives by the screening cytotechnologist. A cytotechnologist in the United States using the PapNet system then rescreened these smears. Slides identified as potentially abnormal using PapNet were then manually rescreened and preliminarily classified. Smears provisionally interpreted as ASCUS or worse then were classified by a US pathologist [13]. Finally, residual cells remaining on the sampler after preparation

of the smear were rinsed in PreservCyt and used to prepare ThinPrep slides in the United States. ThinPrep smears were then screened in the United States by a cytotechnologist and classified by a pathologist in a manner analogous to smears [14]. A second ThinPrep slide was prepared from cases when indicated for technical reasons. Cytologic interpretations were made independently masked to other data using the 1991 Bethesda System [7].

Patients with any of the following results were referred for colposcopy: 1) cytologic interpretation of ASCUS or more using any of the three cytologic exams; 2) a positive cervigram result; or 3) a suspicion of cancer on physical examination. An experienced gynecologist (J.M.) performed the colposcopic examinations and biopsies. Women with histologic CIN 2 or worse on initial biopsies or curettages and those with findings worrisome for CIN 2 or 3 (e.g., cytologic results of HSIL confirmed on review) were treated with loop electrosurgical excision procedure (LEEP). Women were assigned a final enrollment diagnosis reflecting all available data [11, 12]. At the end of enrollment, 119 patients had a histologically confirmed final diagnosis of CIN 2 or 3, constituting the subjects included in this analysis.

Cervigram and Colposcopic Review

The conventional 35-mm slide cervigrams, digitally scanned images of the cervigrams, and reports of colposcopy were reviewed retrospectively by an experienced gynecologist (J.J.) to determine the location of the CIN 2 or 3 lesions. The 35-mm slides of cervigrams were evaluated using a Pradovit RT-m Leica projector, which permitted the projection of a 6-foot image on a flat, white screen. The cervigrams slides also were scanned using the Scanjet ADF scanner (Hewlett Packard, Palo Alto, CA) with slide adapter, stored in JPEG format, and evaluated on a 17-inch color monitor. Cervigrams were unavailable for three patients. Lesion location could not be determined for another five women. Thus, the lesion location was determined for 111 women.

Retrospective reviews of the cervigrams from the 111 women with CIN 2 or 3 were performed in a masked fashion in two batches. The first batch included women with a smear interpreted as negative using all three cytologic methods, and women with manually screened smears interpreted as HSIL (n = 63). The second batch of reviews included all remaining women with CIN 2,3 histologic results (n = 48). To avoid biasing in the second masked review, 73 histologic CIN 1 cases were chosen randomly and were reviewed concurrently. The results of CIN 1 reviews were not included in these analyses.

The lesion location on the ectocervix was classified according to the hours of a clock face. To distinguish between those lesions that were primarily on the anterior and posterior ectocervix from those on the woman's right and left side of the ectocervix, the cervix was divided in three anatomic areas: left (between 1 and 5 o'clock), right (between 7 and 11 o'clock), and midline/endocervical (between 11 and 1 o'clock, 5 and 7 o'clock, and endocervical "canal" lesions; Figure 1). We used this classification to create a buffer (middle/endocervical) for better discrimination between lesions located on the left and right ectocervix. We considered an anatomic area to be "involved" if a lesion occupied any fraction of that area. Therefore, patients could have lesions from one to three areas. Most lesions (106 of 111; 95.5%) that we could evaluate had at least partially involved the midline/endocervical area, and thus this characteristic was not useful for discriminating the location of lesions between patients. Accordingly, lesions were classified either as right-hand positive or negative and left-hand positive or negative (R–/L–, R+/L–, R–/L+, R+/L+). Lesions were also categorized as to whether the lesion was present in the endocervical canal, and whether the canal was the only location of the lesion.

Secondary analyses using a different classification of

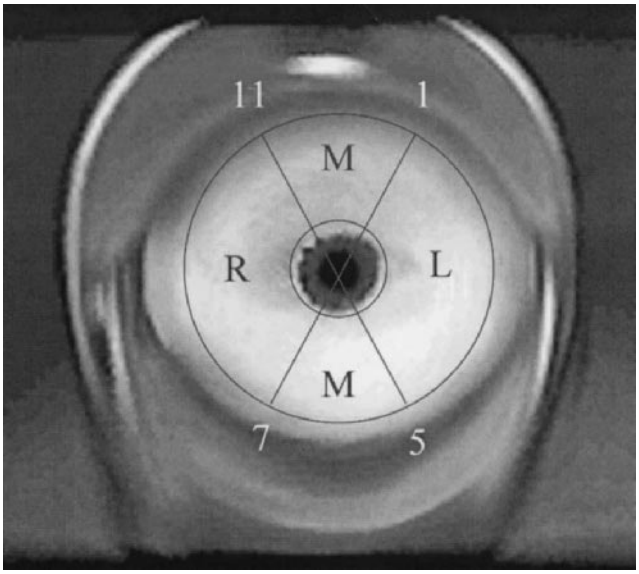

Figure 1. The ectocervix was classified, based on a clock face, as either right (7–11 o'clock), left (1–5 o'clock), or middle (11–1 and 5–5 o'clock and canal).

lesion location were performed to confirm the robustness of our main analyses. A vertical (bisecting) midline of the ectocervix was used to distinguish left and right ectocervix, and lesions were categorized as R+/L–, and R–/L+ or midline/endocervical.

For cervigrams in which no ectocervical lesion was visually apparent, the colposcopy reports were reviewed to confirm the absence of an ectocervical lesion. The colposcopist had completed these forms immediately after finishing the exam to indicate the anatomic location of the lesion by marking a diagram of the cervix.

Human Papillomavirus DNA Detection

Hybrid Capture 2 (HC2) (probe B) testing for HPV DNA of 13 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) was previously performed masked to other results at Digene Corporation [15, 16]. Of 111 cases of CIN 2 or 3, 109 (98.2%) were successfully tested using HC2. The HPV viral load in positive specimens was assessed semiquantitatively as the ratio of light emitted by a specimen to the signal produced by three HPV 16 controls (RLU/PC) containing 1.0 pg/mL HPV DNA (approximately 5,000 viral copies).

Polymerase chain reaction (PCR) testing was performed using MY09/11 L1 consensus primers as detailed elsewhere [17–19]. The PCR products were analyzed by gel electrophoresis and then transferred to nylon filters. The filters were hybridized overnight with a radiolabeled generic probe set for HPV (HPV 11, 16, 18, 51, 73, and 81 combined). Two observers evaluated the signal strength of the PCR products, and these values were used as semiquantitative measures of viral load. All PCR products positive by the generic probe set hybridization were tested by dot blot hybridization with type-specific probes for HPV types: 2, 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42–45, 51–59, 61, 62, 64, 66–70, 71 (AE8), 72, 73, 81 (AE7), 82 (W13B), 83 (PAP291), 84 (PAP155), 85 (AE5), 89 (AE6), AE2 (IS39), AE9, and AE10. Probes for HPV types 2, 13, 34, 42–4, 57, 62, 64, 69, 74, 82 (W13B), and AE9 were used in combination. A specimen was considered HPV positive but uncharacterized if it tested positive for HPV DNA by the generic probe set but not positive for any specific probe. Three experienced investigators interpreted each dot blot result, and discrepancies were resolved by consensus. All 111 patients included in this analysis had available PCR test results.

Statistical Analysis

The relationship of cytologic interpretations determined using the three methods was compared with the lesion location using Pearson χ^2 tests. For direct comparisons of R+/L– lesions to R–/L+ lesions, Fisher exact tests (two-sided) were used because of small numbers of each type of lesion. Cytologic interpretations of ASCUS and LSIL were combined into a single intermediate category (ASC/LSIL).

To examine the association of lesion location with other variables, Kruskal-Wallis tests for continuous variables and Pearson χ^2 tests or Fisher exact tests for categorical variables were used. We evaluated questionnaire variables (current oral contraceptive use, lifetime number of births, and age) and measurements of cervicovaginal microenvironment (cervical inflammation [20] and vaginal pH) as potential confounders of cytologic interpretation.

Kappa statistics and symmetry χ^2 tests were used to evaluate interrater agreement above chance between pathologists interpreting cytologic reviews.

RESULTS

Anatomic Location of Cervical Intraepithelial Neoplasia 2 and 3 Lesions

In 53 cases (48%), cervigrams displayed involvement of one anatomic area; in 23 cases (21%), two areas were involved; and in 35 cases (32%), three areas were involved. These data included 48 patients (43%) with right-sided ectocervical involvement, 50 patients (45%) with left-sided ectocervical involvement, and 106 patients (95%) with midline/ectocervical involvement. The overall classification of the location of the lesions was R–/L– in 50 cases (45%), R+/L– in 11 cases (10%), R–/L+ in 13 cases (12%), and R+/L+ in 37 cases (33%).

Cytologic Interpretations Using Three Techniques

Of the 109 manually screened smears that were satisfactory for interpretation, 26 (24%) were interpreted as negative or benign reactive changes as compared with 14 of 88 (15.9%) evaluated with PapNet. Of 109 Thin Prep slides, 10 (9.2%) were classified as negative or benign reactive changes. Pair-wise agreement between the three cytologic techniques ranged from 57% to 59%, with κ values between 0.21 and 0.31. ThinPrep cytologic analysis was more likely to be interpreted as more severe than conventional Pap ($p = .004$, symmetry χ^2) and PapNet ($p = .04$, symmetry χ^2).

Comparison of Cytologic Interpretations and Location of Cervical Intraepithelial Neoplasia 2 or 3

Table 1 compares the cytologic interpretations reported with the three methods to the location of the CIN 2 or 3 lesions. The cytologic classification of manually screened smears varied significantly with the location of the lesions ($p < .001$, Pearson χ^2); lesions classified as R+/L– were more frequently associated with negative interpretations than those classified as R–/L+ ($p < .001$, Fisher exact test; Table 1A). PapNet results also showed similar variation with regard to lesion location and were more often negative in association with R+/L– lesions than with R–/L+ lesions, but these comparisons were not statistically significant ($p = .2$, Pearson χ^2, overall; $p = .3$, Fisher exact test, R+/L– vs R–/L+; Table 1B). Similarly to manually screened smears, ThinPrep results varied significantly with the location of the lesion ($p < .001$, Pearson χ^2), and lesions classified as R+/L– were more frequently associated with negative interpretations than those classified as R–/L+ ($p = .006$, Fisher exact test; Table 1C). Using the most severe interpretation of the three cytologic reviews (Table 1D), a similar pattern was observed, as R+/L– was more likely to be called cytologically negative than R–/L+ ($p = .004$, Fisher exact test); five of the eight lesions that were associated with negative results using all three cytologic techniques were R+/L–. Lesions classified as R+/L– also were more likely to have at least one cytologic negative result than any other anatomic classification ($p = .002$, Pearson χ^2, overall; $p = .001$, Fisher exact test, R+/L– vs R–/L+; data

Table 1. Comparisons of Cervical Intraepithelial Neoplasia 2,3 Lesion Location as Determined by Cervigram Review Versus Cytologic Result

	Lesion location				
	R−/L−	R+/L−	R−/L+	R+/L+	Total
A[a]					
Cytologic negative	8	8	1	9	26
	16.0%	88.9%	7.7%	24.3%	23.9%
ASC/LSIL	6	0	3	7	16
	12.0%	0.0%	23.1%	18.9%	14.7%
HSIL	36	1	9	21	67
	72.0%	11.1%	69.2%	56.8%	61.5%
Total	50	9	13	37	109
B[b]					
Cytologic negative	3	3	1	7	14
	8.1%	42.9%	8.3%	21.9%	15.9%
ASC/LSIL	13	2	6	12	33
	35.1%	28.6%	50.0%	37.5%	37.5%
HSIL	21	2	5	13	41
	56.8%	28.6%	41.7%	40.6%	46.6%
Total	37	7	12	32	88
C[c]					
Cytologic negative	3	5	0	2	10
	6.0%	55.6%	0.0%	5.4%	9.2%
ASC/LSIL	13	2	3	12	30
	26.0%	22.2%	23.1%	32.4%	27.5%
HSIL	34	2	10	23	69
	68.0%	22.2%	76.9%	62.2%	63.3%
Total	50	9	13	37	109
D[d]					
Cytologic negative	1	5	0	2	8
	2.0%	45.5%	0.0%	5.4%	7.2%
ASC/LSIL	4	2	1	5	12
	8.0%	18.2%	7.7%	13.5%	10.8%
HSIL	45	4	12	30	91
	90.0%	36.4%	92.3%	81.1%	82.0%
Total	50	11	13	37	111

ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; R−, right-side negative lesion; R+, right-side positive lesion; L−, left-side negative lesion; L+, left-side positive lesions.
A Costa Rican pathologist (A) and then a U.S. pathologist (B) reviewed Pap smears. ThinPrep slides, produced from the residual specimen used to make Pap smears, were interpreted by third pathologist (C). Finally, using the worst cytologic interpretation of the three reviewed, the data were combined (D). Column percentages are shown in italics.
[a] $p < .001$, Pearson χ^2, overall; $p < .001$, Fisher exact test, R+/L− vs R−/L+.
[b] $p = .2$, Pearson χ^2, overall; $p = .3$, Fisher exact test, R+/L− vs R−/L+.
[c] $p < .001$, Pearson χ^2, overall; $p = .006$, Fisher exact test, R+/L− vs R−/L+.
[d] $p < .001$, Pearson χ^2, overall; $p = .004$, Fisher exact test, R+/L− vs R−/L+.

not shown). A histologic and cytologic summary of the 11 lesions classified as R+/L– is shown in Table 2. Results from secondary analyses that categorized lesions as either midline/endocervical, right, or left were similar (data not shown).

Comparison of Human Papillomavirus Test Results and Anatomic Location of Cervical Intraepithelial Neoplasia 2 or 3

We compared the HPV DNA testing of the exfoliative cells specimens by HC2 [13] and MY09/11 PCR [14] with lesion location (Table 3). We did not observe significant differences in HPV detection by lesion location for either HC2 ($p = .5$, Pearson χ^2) or for MY09/11 PCR ($p = .9$, Pearson χ^2). Viral load for R+/L– (median, 18.5 RLU/PC) was nonsignificantly less than for the other categories (≥64.9 RLU/PC; $p = .2$, Kruskal-Wallis, overall; $p = .2$, Kruskal-Wallis, R+/L– vs. R–/L+). A similar, marginally significant pattern was observed for the PCR signal strength. Only 30% of the R+/L– lesions had signal strength scores of more than 3 (scale, 1–5). By contrast, more than 70% of those lesions with other topographical distributions were scored as above 3 ($p = .01$, Pearson χ^2, overall; $p = .08$, Fisher exact test, R+/L– vs R–/L+). The two measures of HPV viral load were highly correlated ($p = .0001$, Kruskal-Wallis).

Comparison of Demographics and Risk Factors for Cervical Cancer and Anatomic Location of Cervical Intraepithelial Neoplasia 2 or 3

Finally, we evaluated the relationship of lesion location with several covariates that may influence the performance of cytologic analysis (Table 4). Overall, there was a significant difference in age by lesion location ($p = .0004$, Kruskal-Wallis), but this was primarily the result of women with R–/L– (middle only) being older (median age, 38 years), and there was nonsignificant difference in the age of women with R+/L– (median age, 30 years) and R–/L+ (median age, 33 years; $p = .2$, Kruskal-Wallis). There was no apparent difference in current oral contraceptive use by lesion location. There were overall differences in the percent of women with vaginal pH > 4.5 ($p = .03$, Pearson χ^2), an indicator of bacterial vaginosis, but there were no differences in the percentage with vaginal pH > 4.5 between women with R+/L– and R–/L+ lesions ($p = .5$, Fisher exact test). There were also overall differences in the percent of women with cervicitis (>30 neutrophils/field of view; $p = .006$, Pearson χ^2), and the percentage among women with R–/L+ lesions (84.6%) was much greater than among women with R+/L– lesions (25.0%; $p = .02$, Fisher exact test). Of the several variables assessed by questionnaire, women with R+/L– lesions had fewer pregnancies (median, 2) than those with R–/L+ lesions (median, 4; $p = .02$, Fisher exact test).

Table 2. Cytologic Results and Details of Lesion Location of the 11 Women with Right Ectocervix Cervical Intraepithelial Neoplasm 2, 3 Lesions

	Conventional Pap					
	Costa Rican pathologist	US pathologist[a]	ThinPrep	Worst cytology	Any negative cytology	Lesion location (o'clock)
1		ASC/LSIL	HSIL	HSIL	No	5 to 10
2	Negative		Negative	Negative	Yes	10 to 12
3		Negative	Negative	Negative	Yes	10 to 1
4	Negative		Negative	Negative	Yes	9 to 12
5	Negative		HSIL	HSIL	Yes	6 to 9
6	Negative	HSIL	Negative	HSIL	Yes	5 to 6 & 10 to 12
7	HSIL	HSIL	ASC/LSIL	HSIL	No	9 to 1
8	Negative	ASC/LSIL		ASC/LSIL	Yes	5 to 8
9	Negative	Negative		Negative	Yes	7 to 11
10	Negative		ASC/LSIL	ASC/LSIL	Yes	9 to 12
11	Negative	Negative	Negative	Negative	Yes	6 to 12

ASC, atypical squamous cells; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.
[a]Assisted using PapNet.

Table 3. Comparisons of Cervical Intraepithelial Neoplasia 2, 3 Lesion Location as Determined by Cervigram Review versus Human Papillomavirus DNA Testing by Hybrid Capture 2 and MY09/11 L1 Consensus Primer Polymerase Chain Reaction

	Lesion location					
	R−/L− (*n* = 50)	R+/L− (*n* = 11)	R−/L+ (*n* = 13)	R+/L+ (*n* = 37)	p^a	p^b
Hybrid Capture 2						
HPV+, *n* (%)	43 (89.6%)[c]	8 (72.7%)	11 (84.6%)	32 (86.2%)	.5	.6
RLU/PC, median (range)[d]	70.2 (1.4–1,335.1)	18.5 (1.6–223.4)	64.9 (7.5–926.5)	136.1 (1.4–1602.1)	.2	.2
MY09/11 PCR						
HPV+, *n* (%)	43 (86.0%)	10 (90.9%)	12 (92.3%)	33 (89.2%)	.9	1.0
Signal strength >3, %[d]	81.4%	30.0%	75.0%	72.7%	.01	.08

R−, right-side negative lesion; R+, right-side positive lesion; L−, left-side negative lesion; L+, left-side positive lesion; HPV, human papillomavirus; PCR, polymerase chain reaction.
RLU/PC from Hybrid Capture 2 and signal strength from PCR were used as semiquantitative measures of viral load.
[a]Kruskal-Wallis test (continuous variable) and Pearson χ^2 (categorical variable).
[b]Compares R+/L− to R−/L+ using Kruskal-Wallis test (continuous variable) and two-sided Fischer exact test (categorical variable).
[c]Two women with R−/L− lesions did not have Hybrid Capture 2 testing.
[d]Restricted to test positive specimens.

DISCUSSION

Our results suggest that lesions located on the right ectocervix may be more likely to be poorly sampled using a Cervix brush than those located on the left. As a consequence, these lesions may be more likely to be missed by cytologic screening. We stress that this conclusion is tentative, based on small numbers of missing high-grade lesions and a single cell collection protocol. The finding should be corroborated before the conclusions are accepted. However if true, although only 10% of CIN 2 or 3 lesions were located exclusively on the right ectocervix, suboptimal sampling of this anatomic area of the cervix could lead to avoidable screening failures.

Three observations support our proposed conclusion. First, using two different cytologic techniques, a conventional Pap smear and a ThinPrep slide, lesions classified as R+/L– were significantly more frequently interpreted as negative than those classified as R–/L+. Results using PapNet, a semiautomated, neural network-based device capable of facilitating the identification of false-negative smears [13], also revealed a nonsignificant increase in false-negative results with R+/L– lesions. Second, although there were no differences in HPV detection by lesion location as may be expected with highly sensitive assays, HPV viral load measured independently by HC2 and PCR on a second collected specimen was lower for R+/L– lesions than for other topographical locations. The HPV specimen was taken with a separate swab. We postulate that much of the cellular material collected by the swab was exfoliated by the preceding scraping using the Cervex broom. This would explain why viral load may be associated with ectocervical position concordant with the cytologic findings. Finally, a different categorization of lesion location did not alter the association of suboptimal cytologic performance with right ectocervical lesion location, supporting the robustness of the finding.

Table 4. Comparisons of Cervical Intraepithelial Neoplasia 2, 3 Lesion Location as Determined by Cervigram Review versus Select Characteristics

		Lesion location					
	n	R−/L−	R+/L−	R−/L+	R+/L+	p^a	p^b
Age (y)	111	38	30	33	31	.0004	.2
Current OC user	91	13/37 (35.1%)	5/11 (45.5%)	8/13 (61.5%)	11/37 (36.7%)	.4	.7
Vaginal pH >4.5	111	23/50 (46.0%)	2/11 (18.2%)	1/13 (7.7%)	11/37 (29.7%)	.03	.5
Have cervicitis	79	18/28 (64.3)	2/8 (25.0%)	11/13 (84.6%)	11/30 (36.7%)	.006	.02
Median no. of pregnancies	109	5	2	4	4[c]	.01	.009

R−, right-side negative lesion; R+, right-side positive lesion; L−, left-side negative lesion; L+, left-side positive lesion. OC, oral contraceptive.
Cervicitis was determined by averaging the counts white blood cells on Pap smears in five nonadjacent microscope fields and any women with an average count exceeding 30 was considered to have cervicitis.
[a]Kruskal-Wallis test (continuous variable) and Pearson χ^2 (categorical variable).
[b]Compares R+/L− to R−/L+ using Kruskal-Wallis test (continuous variable) and two-sided Fischer exact test (categorical variable).
[c]Two women with R+/L+ lesions had never been pregnant.

We confirmed with the nurses who collected the specimens that they followed the sampling technique according to the manufacturer's recommended procedures for the collection of cervical specimens. Although the nurses were trained by a US gynecologist [11] and have collected more than 10,000 Pap smears, inconsistencies in sample technique used to collect the sample may explain our results. We offer three hypotheses to explain our finding. First, each clockwise rotation was incomplete and therefore failed to sample the right ectocervix as thoroughly as the left ectocervix. A second hypothesis is that greater pressure was inadvertently applied to the left side when collecting a sample, leaving the right side inadequately sampled. A third possibility is that if the right ectocervical area is the last to be sampled, lesions may be more poorly sampled because of saturation of the collection device with cells. Finally, the distributions of lesions with particular characteristics may have differed by chance, leading again to a need to replicate these findings in another study population.

We evaluated the influence of handedness on Pap smear performance, and our results suggest that right handedness is more likely to result in missed right-side lesions than left handedness ($p = .1$). This finding would support our hypothesis about most clinicians inadvertently applying more pressure on the left side of the cervix during rotation of the sampler. Because our results are based on a small number of patients and we did not find a statistical difference, we recommend additional studies to explore handedness.

In fact, there was some evidence that the right-sided lesions may be intrinsically different. There were significant differences in the percentage of each classification having endocervical canal involvement, primarily as the result of inclusion of the endocervical-only lesions with the R–/L– classification (20 of 50; 40%), and there was also a greater tendency for R+/L– to have endocervical involvement than R–/L+ ($p = .08$, Fisher exact test). Yet the R–/L– had a similar false-negative proportion and viral load as R–/L+, suggesting that endocervical lesions were effectively sampled and did not explain the cytologic misses and lower viral loads found for R+/L–.

Influence of lesion size was not evaluated and thus could explain our findings. A recent report demonstrated that the size of CIN 3 lesions is an important determinant for cytologic detection [21]. Subsequent studies of lesion location will need to incorporate this measurement to assess its effect. Currently, we are developing digital methods to measure lesion size while correcting for the orientation of the cervix.

We examined a number of factors that may confound the performance of cytologic analysis. Both number of pregnancies and vaginal pH were strongly associated with age (<30 years, 30–39 years, 40 or more years; $p < .001$, Pearson χ^2) and differences between groups may simply reflect age differences, with the R–/L– group being much older than other groups. We could not assess time since last pregnancy, and women with R+/L– lesions were younger and therefore might have been more recently pregnant. Reduced number of pregnancies among women with R+/L– lesions may also be related to increased number of endocervical lesions, but as discussed above, this factor does not appear to explain our main finding. Cervicitis and bacterial vaginosis (as suggested by higher vaginal pH) can produce cytologic changes classified as ASCUS, which may lead serendipitous detection of CIN 2 or 3. These finding were less common in R+/L– lesions and therefore may explain the differences in the rates of cytologic negatives between groups. Conversely, such factors may also obscure the identification of abnormal cells. It is noteworthy that the difference in cytologic detection (i.e., an interpretation of HSIL) between R+/L– and R–/L+ for detection of histologic CIN 2,3 was still significant when combining the cytologically negative with the ASC/LSIL interpretations using the combined cytologic data ($p = .004$, Fisher exact test) or combining the ASC/LSIL with HSIL ($p = .006$, Fisher exact test).

We conclude that lesion location may influence the performance of cytologic screening, but a larger study is needed to confirm this finding. Because of small numbers, we cannot rule out a chance finding. A recent study found that lesions with abnormally high expression of E-cadherin may lead to false-negative cytologic results among patients with cervical neoplasia, perhaps because of poorer exfoliation of cervical epithelial cells [22]. The E-cadherin finding, the effect of lesion size, and our technical observation are not mutually exclusive. A formal analysis of these factors is needed to assess their relationships and to determine the fraction of false-negative cytologic results that are attributable to them. If our findings are confirmed, clinicians using broom-type single-specimen samples should have a protocol for assuring to collect samples from the full face of the cervix with adequate pressure.

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